

PATENT
1912-151P

IN THE U.S. PATENT AND TRADEMARK OFFICE

APPLICANT: Michel IBEA et al
SERIAL NO.: 08/702, ~~113~~ 113 GROUP:
FILED: August 23, 1996 EXAMINER:
FOR: CHIMERIC FATTY BODY-GRF ANALOGS WITH
INCREASED BIOLOGICAL POTENCY

DECLARATION UNDER 37 C.F.R. 1.132

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I Dr. Paul Brazeau of 4054 Avenue du Parc Lafontaine,
Montréal, Québec, Canada do hereby declare the following.

I am co-inventor of the above-captioned application. As such,
I am fully knowledgeable of the present invention and the disclosure
of the specification.

As evidenced by my curriculum vitae, a copy of which is
enclosed herewith, I am considered as one-skilled in the art of the
present invention. I am also fully knowledgeable of the references
of Gaudreau et al., Coy et al., Felix et al., Bercu et al., Kann et
al., Recker and Clark, as cited by the Examiner.

The comparative experiments presented below, which were
conducted under my direct supervision and control, demonstrate the
unexpected and unobvious advantages over the compounds of Gaudreau
et al. and Coy et al. There is further no suggestion of these
advantages in the remaining cited references.

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Introduction

The present invention is drawn to a chimeric fatty body-pro-GRF analog with increased biological potency, of the following general formula:

A1-A2-Asp-Ala-Ile-Phe-Thr-A8-Ser-Tyr-Arg-Lys-
Val-Leu-A15-Gln-Leu-A18-Ala-Arg-Lys-Leu-Leu-
A24-Asp-Ile-A27-A28-Arg-A30-R₀

wherein,

A1 is Tyr or His;

A2 is Val or Ala;

A8 is Asn or Ser;

A15 is Ala or Gly;

A18 is Ser or Thr;

A24 is Gln or His;

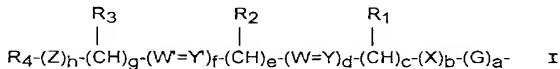
A27 is Met, Ile or Nle;

A28 is Ser or Asp;

A30 is any amino acid sequence of 1 to 15 residues;

R₀ is NH₂;

wherein A1 is N- or O-anchored by a hydrophobic tail of the following general formula I:



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wherein,

G is a carbonyl, a phosphonyl, a sulfuryl or a sulfinyl group;

X is an oxygen atom, sulfur atom or an amino group (NH);

(W=Y) represents cis or trans ($\text{CH}=\text{CR}_5$);

(W'=Y') represents cis or trans ($\text{CH}=\text{CR}_6$);

Z is an oxygen or a sulfur atom;

R₁, R₂ and R₃, independently, are selected from a hydroxyl group, a hydrogen atom, and a linear or branched C₁-C₆ alkyl group;

R₄ is a hydroxyl group, a hydrogen atom or a linear or branched C₁-C₆ alkyl group;

R₅ and R₆, independently, are a hydrogen atom or a linear or branched C₁-C₄ alkyl group;

a is 0 or 1;

b is 0 or 1;

c is 0 to 8;

d is 0 or 1;

e is 0 to 8;

f is 0 or 1;

g is 0 to 8;

h is 0 to 1;

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wherein the sum of $d + f \geq 1$ and the sum of a, b, c, d, e, f, g and h is such that the hydrophobic tail of formula I has a linear main chain of between 5 and 7 atoms (C, O and/or S).

Thus, the present invention is drawn to alkene based analogs of chimeric fatty body-pro-GRF compounds having a linear main chain of between 5-7 carbons.

Experimental procedures

Experiment 1 - Superior activity of alkene based main chains

Methods:

Thirty-two (32) pigs were randomly distributed into 4 experimental groups ($n = 8$ pigs per group). Each group received two daily S.C. administrations of the following treatments (volume: 3 ml, S.C. injection).

- group 1: saline
- group 2: (Hexanoyl)₀ hGRF(1-29)NH₂
- group 3: (Hexenoyl *trans*-2)₀ hGRF(1-29)NH₂
- group 4: (Hexenoyl *trans*-3)₀ hGRF(1-29)NH₂

Treatments were administered from day 1 to 5. Immediately before the injections, one blood sample was collected from each animal, and additional blood samples were collected on days 6, 7 and 8 in group 2 only.

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Blood samples were allowed to clot, serum was harvested by centrifugation and sent to Notre-Dame Hospital for IGF-I assays.

Results:

As seen in Table I and Figures 1 and 2, hexenoyl compounds (both *trans*-2 and *trans*-3) remain active much longer than the corresponding hexanoyl compound, as evidenced by the serum concentrations of IGF-1.

Experiment 2 - Effects of linear chain main chain length

Thirty two (32) pigs were cannulated (a catheter surgically implanted in one jugular vein) within one week before the study. Cannulated animals were randomly distributed into 4 experimental groups (n = 8 pigs per group).

group 1: hGRF(1-29)NH₂
group 2: (Octanoyl)₀ hGRF(1-29)NH₂
group 3: (Hexanoyl)₀ hGRF(1-29)NH₂
group 4: (Butyryl)₀ hGRF(1-29)NH₂

All groups were subjected to the following sequence of events:

Day 1: single IV administration (4 µg/kg)
Blood samples for pGH assay were collected every 20 min from 1 hour before to 5 hours after test articles injections, with additional samples 10 and 30 min after injection (n = 21 samples per profile).

From day 8 to day 12, twice daily SC administration of test articles (20 µg/kg) to all animals:

day 8: SC injection at 9:00 am and 6:00 pm
day 9: SC injection at 9:00 am and 6:00 pm
day 10: SC injection at 9:00 am and 6:00 pm
day 11: SC injection at 9:00 am and 6:00 pm

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day 12: SC injection at 9:00 am and 6:00 pm

Blood samples for pGH assay were collected on day 8 (first injection of test articles) from 1 hour before to 6 hours after test article injections (every 20 min; n = 22 samples per profile).

Additional blood samples were collected on days 9, 10, 11, 12, 13 (before test article administration) for IGF-I measurement.

Blood samples were allowed to clot overnight at 4°C. Serum was harvested by centrifugation, stored at -20°C and sent to Notre-Dame Hospital for pGH and IGF-I assays.

Results:

As seen in Table 2 and Figure 3, chimeric fatty body-pro-GRF compounds having a linear main chain of 6 carbons have unexpected superior activity over compounds with either a shorter (butyryl = 4 carbons) or a longer (octanoyl = 8 carbons) main chain length, as evidenced by the serum concentration of IGF-1.

Conclusions

Neither Gaudreau et al. nor Coy et al. disclose fatty body-pro-GRF compounds having a double bond and a linear main chain of between 5-7 carbons. Gaudreau et al. disclose alkane based compounds of 3, 4, 6 or 8 carbons. However, in Gaudreau et al. there is no suggestion of alkene based compounds or any advantages of compounds having 5 to 7 carbons. In addition, Coy et al. only

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disclose acetyl based compounds.

The experiments described herein clearly demonstrate that alkane fatty body pro-GRF compounds having 5 to 7 carbons in the main chain have unexpected, unobvious activity over those of disclosed by Gaudreau et al. and Coy et al.

As such, in my opinion the superior activity and advantages of the compounds of the present invention are in no way disclosed or suggested by the cited prior art references and the present invention is therefore unobvious over the prior art.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

This 15th day of September, 1996


Dr. Paul Bratton

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This 15th day of September, 1996


Dr. Paul Brasead